PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	R.P. Nargund et al.		
Serial No.:	10/730,704	Case No.: 21151	Art Unit: 1614
Filed:	December 8, 2003		Examiner: Phyllis G. Spivack
For:	ACYLATED PIPERAZINE DERIVATIVES AS MELANOCORTIN -4 RECEPTOR AGONISTS		

Mail Stop AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

Sir:

I, Dr. Alison Merwin Strack hereby declare:

1. I am currently employed by Merck & Co., Inc. as a Senior Research Fellow, and have been employed as a scientist at Merck & Co., Inc. since 1997. Part of my responsibilities at Merck Research Laboratories at Merck & Co., Inc. include work on projects involving the pharmacology of obesity and metabolic disorders, including the design and interpretation of *in vivo* pharmacology research in testing compounds for their use to treat obesity and metabolic syndrome.

2. My educational background is as follows:

University of California, Department of Physiology 1990 to 1995
Postdoctoral Fellow (sponsor, Mary F Dallman, Ph.D.) Research on interactions between sympathetic nervous system and glucocorticoid regulation of metabolism and of the hypothalamic pituitary adrenal axis; hypothalamic regulation of feeding.

Ph.D., Washington University, St. Louis 1990
Graduate Program in Neural Sciences. (sponsor Arthur Loewy, Ph.D.) Thesis title: "CNS Sites Controlling the Sympathetic Nervous System Determined with a Viral Transneuronal Marker"

B.S., Biology University of Michigan 1985.

3. My academic experience is as follows:

Research Associate, University of California, San Francisco Physiology Department, 1995-1997.

4. My employment experience is as follows:

Merck Research Laboratories, Department of Pharmacology

Jan 2005 to present	Senior Research Fellow
Feb 2002 to Dec 2004	Research Fellow
Sept 1997 to Jan 2002	Senior Research Biologist

5. My honors and awards are as follows:

- MRL Process Optimization Top 10 Finalist as member of the Metabolic Rate Measurement Team, 2004
- Awarded (and declined $1,021,801) RO1 from NIMH for grant titled Hypothalamic 5-HT2C receptor control of energy balance, 1997.
- American Heart Association, California Affiliate Postdoctoral Fellow, University of California San Francisco, 1993 to 1995
- University of California Tobacco-Related Disease Research Program Postdoctoral Fellow, University of California San Francisco, 1991 to 1993
- 13th James L.O'Leary Prize for Research in Neuroscience, Washington University School of Medicine, St. Louis, Missouri, 1990
- National Merit Scholar, University of Michigan, Ann Arbor, Michigan, 1981 to 1985
- University of Michigan Academic Recognition Scholarship, University of Michigan, Ann Arbor, Michigan, 1981

6. My society memberships are as follows:

American Diabetes Association
American Physiological Society
2005 Member of Neural Control and Autonomic Regulation Steering Committee

International Society for Neuroendocrinology
International Society for the Autonomic Nervous System
North American Association for the Study of Obesity
Society for Neuroscience

1993-present Manuscript Referee: *J. Neuroscience, Neuroscience, J. Comp. Neurology, J. Auton. Nervous System, Brain Research, Am. J. Physiology, J. Clin. Invest., Neuroendocrinol.*

7. My presentations are as follows:

1) Neural Regulation of Cardiovascular Control, FASEB Summer Conference, Saxton River, VT, June 11-15, 1990.

2) Neuroanatomical Methodology: The Herpes Viruses--Probes for the Study of Neuronal Connectivity and Function, Society for Neuroscience, St. Louis, MO, Oct. 28-Nov. 2, 1990.

3) Gene Transfer into Neurones: From Basic Applications to Gene Therapy, Cardiff Neuroscience International Symposium, Cardiff, Wales, August 16-18, 1993.

4) Energy Balance: Glucocorticoids, Insulin & Serotonin. Amgen, Inc., Thousand Oaks, CA, Oct. 25, 1996.

5) Neural and Endocrine Regulation of Temperature and Energy Balance. Univ. Texas Health Science Center, San Antonio, TX, Dec. 19, 1996.

6) Energy Balance: Glucocorticoids, Insulin & Serotonin. Genentech, South San Francisco, CA Feb. 20, 1997.

7) Energy Balance: Glucocorticoids, Insulin & Serotonin. Merck & Co., Rahway, NJ.,Apr. 3, 1997.

8) CNS Regulation of Energy Balance. Chair and speaker of symposium at the Annual Meeting of Society for Neuroscience, Los Angeles, Nov. 1998.

9) Obesity Pharmacotherapy: CNS Targets. IBC International Conference on Metabolic Syndrome, Boston, MA, Aug 6-8, 2003.

10) Melanocortin Agonists as Potential Anti-obesity Therapeutics. CBI Therapeutic Approaches to Obesity and Related Disorders Conference, Washington, DC, July 21-22, 2005.

11) Basic Research and Obesity Therapeutics: Finding a Good Target, Alberta Obesity Summit, Kannanaskis Village, Alberta, May 8-9, 2006.

12) The identification of CNS targets for obesity therapeutics, SMi Metabolic Diseases Forum, London, England, October 2-3, 2006.

8. My publications are as follows:

1) Sessler FM, Jokelainen PT, Sing CF, Strack AM, Malvin RL. Renin heterogeneity in stroke-prone hypertensive and normotensive rats. Am J Physiol. 1986;251(4 Pt 1):E367-72.
2) Strack AM, Sawyer WB, Marubio LM, Loewy AD. Spinal origin of sympathetic preganglionic neurons in the rat. Brain Res. 1988;455(1):187-91.
3) Strack AM, Sawyer WB, Hughes JH, Platt KB, Loewy AD. A general pattern of CNS innervation of the sympathetic outflow demonstrated by transneuronal pseudorabies viral infections. Brain Res. 1989;491(1):156-62.
4) Strack AM, Sawyer WB, Platt KB, Loewy AD. CNS cell groups regulating the sympathetic outflow to adrenal gland as revealed by transneuronal cell body labeling with pseudorabies virus. Brain Res. 1989;491(2):274-96.
5) Strack AM, Loewy AD. Pseudorabies virus: a highly specific transneuronal cell body marker in the sympathetic nervous system. J Neurosci. 1990;10(7):2139-47.
6) Akana SF, Dallman MF, Bradbury MJ, Scribner KA, Strack AM, Walker CD. Feedback and facilitation in the adrenocortical system: unmasking facilitation by partial inhibition of the glucocorticoid response to prior stress. Endocrinology. 1992;131(1):57-68.
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8) Dallman, M.F., S.F. Akana, K.A. Scribner, M.J. Bradbury, C.-D. Walker, A.M. Strack, and C.S. Cascio 1992 Stress, feedback and facilitation in the hypothalamo-pituitary-adrenal axis. *J. Neuroendocrinol.*, 4:517-526, 1992.
9) Schramm LP, Strack AM, Platt KB, Loewy AD. Peripheral and central pathways regulating the kidney: a study using pseudorabies virus. Brain Res. 1993;616(1-2):251-62.
10) Bradbury, MJ, AM Strack, and MF Dallman. Lesions of the hippocampal efferent pathway (fimbria-fornix) do not alter sensitivity of adrenocorticotropin to feedback inhibition by corticosterone in rats. Neuroendocrinol. 1993; 58 (4): 396-407.
11) Hanson ES, Bradbury MJ, Akana SF, Scribner KS, Strack AM, Dallman MF. The diurnal rhythm in adrenocorticotropin responses to restraint in adrenalectomized rats is determined by caloric intake. Endocrinology. 1994; 134(5):2214-20.
12) Akana SF, Strack AM, Hanson ES, Dallman MF. Regulation of activity in the hypothalamo-pituitary-adrenal axis is integral to a larger hypothalamic system that determines caloric flow. Endocrinology. 1994;135(3):1125-34.
13) Dallman, M.F., S.F. Akana, M.J. Bradbury, A.M. Strack, E.S. Hanson, and K.A. Scribner. Regulation of the hypothalamo-pituitary-adrenal axis during stress: feedback, facilitation and feeding. *Sem. Neurosci.*, 6: 205-213, 1994.

14) Strack AM, Horsley CJ, Sebastian RJ, Akana SF, Dallman MF. Glucocorticoids and insulin: complex interaction on brown adipose tissue. Am J Physiol. 1995;268(5 Pt 2):R1209-16.
15) Santana P, Akana SF, Hanson ES, Strack AM, Sebastian RJ, Dallman MF. Aldosterone and dexamethasone both stimulate energy acquisition whereas only the glucocorticoid alters energy storage. Endocrinology. 1995;136(5):2214-22.
16) Tecott LH, Sun LM, Akana SF, Strack AM, Lowenstein DH, Dallman MF, Julius D. Eating disorder and epilepsy in mice lacking 5-HT2c serotonin receptors. Nature. 1995;374(6522):542-6.
17) Tkacs NC, Strack AM. Systemic endotoxin induces Fos-like immunoreactivity in rat spinal sympathetic regions. J Auton Nerv Syst. 1995;51(1):1-7.
18) Strack AM, Bradbury MJ, Dallman MF. Corticosterone decreases nonshivering thermogenesis and increases lipid storage in brown adipose tissue. Am J Physiol. 1995;268(1 Pt 2):R183-91.
19) Strack AM, Sebastian RJ, Schwartz MW, Dallman MF. Glucocorticoids and insulin: reciprocal signals for energy balance. Am J Physiol. 1995;268(1 Pt 2):R142-9.
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21) Akana SF, Hanson ES, Horsley CJ, Strack AM, Bhatnagar S, Bradbury MJ, Milligan ED, Dallman MF. Clamped Corticosterone (B) Reveals the Effect of Endogenous B on Both Facilitated Responsivity to Acute Restraint and Metabolic Responses to Chronic Stress. Stress. 1996; 1(1):33-49.
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23) Tkacs NC, Li J, Strack AM. Central amygdala Fos expression during hypotensive or febrile, nonhypotensive endotoxemia in conscious rats. J Comp Neurol. 1997;379(4):592-602.
24) Strack AM, Akana SF, Horsley CJ, Dallman MF. A hypercaloric load induces thermogenesis but inhibits stress responses in the SNS and HPA system. Am J Physiol. 1997;272(3 Pt 2):R840-8.
25) Nonogaki K, Strack AM, Dallman MF, Tecott LH. Leptin-independent hyperphagia and type 2 diabetes in mice with a mutated serotonin 5-HT2C receptor gene. Nat Med. 1998;4(10):1152-6.
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36) Zhou, D, Z Shen, AM Strack, DJ Marsh, and LP Shearman. Enhanced running wheel activity of both *Mch1r*$^{-}$ and *Pmch*-deficient mice. Regul Pept. 2005; 124(1-3):53-63.

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piperidinyl]-1-[(4-fluorophenyl)methyl]-2-oxoethyl]-4-methyl-2-piperazinecarboxamide (MB243), a Potent and Selective Melanocortin Subtype-4 Receptor Agonist. Bioorganic & Medicinal Chemistry Letters, 2005; 15, 171-175.

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45) Shearman, LP, Wang SP, Helmling S, Stribling DS, Mazur P, Ge L, Wang L, Klussmann S, Macintyre DE, Howard AD, Strack AM. (2006) Ghrelin Neutralization by an RNA-Spiegelmer Ameliorates Obesity in Diet-Induced Obese Mice. Endocrinol. 2005 Dec 8; [Epub ahead of print]. 147(3): 1517-1526 (2006).

46) Zhang, J, A Frassetto, R-RC Huang, J Lao, A Pasternak, S-p Wamg. JM Metzger, AM Strack, TM Fong, and RZ Chen. The μ-opioid receptor subtype is required for the anorectic effect of an opioid antagonist. European Journal of Pharmacology 545: 147-152 (2006).

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51) Shearman, LP, Stribling, DS, Camacho, RE, Rosko, KM, Wang, J, Tong, S, Feng, Y, Marsh, DJ, Yu, H, Guan, X, Spann, SK, MacNeil, DJ, Fong, TM, Metzger, JM, Goulet, MT, Hagmann, WK, Plummer, CW, Finke, PE, Mills, SG, Shah, SK, Truong, Q, Van der Ploeg, LHT, MacIntyre, DE and Strack, AM. Characterization of a novel and selective cannabinoid CB1 receptor inverse agonist in rodents (submitted).

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9. I am familiar with Claims 49-54 of Merck's pending patent application U.S.S.N 10/730,704. I have discussed this patent application and Claims 49-54 of this patent application with the patent attorney of record.

10. The *in vivo* studies performed on the combination of AM251 and phentermine described in attached Exhibits 1, 2, 3 and 4 were conducted under my direction. Protocols for the studies and results are discussed herein.

11. Exhibits 1, 2 and 3 are graphs showing the effect of the combination of AM251 (1 mg/kg or 3 mg/kg) and phentermine on body weight and food intake. The data for Exhibits 1, 2 and 3 were obtained as follows:

Study Protocol: *Animals:* 6 month old male diet induced obese (DIO) C57BL/6N mice weighing 47.5 g (36% body fat) at the start of the study were fed with high fat diet and individually housed in a room with a 12 hr light photoperiod. Age-matched lean mice with regular chow were included as controls. Mice were allowed ad libitum access to water and food. *Method:* Mice were conditioned orally with vehicle (5% tween, 0.25% methylcellulose) for 10 days before they were dosed with the drugs listed in Groups 1–7 below. Food intake and body weight change were monitored daily. On day 7, baseline glucose levels were determined. On day 14, serum and plasma were taken for PK and hormone and serum chemistry measurements; fat pads (epididymal, retroperitoneal, mesenteric) and liver were weighed.

Groups (n = 8)

1) Vehicle
2) AM 251 @ 1 mg/kg
3) AM 251 @ 3 mg/kg
4) Phentermine @ 10 mg/kg
5) AM 251 (1) + Phentermine (10)
6) AM 251 (3) + Phentermine (10)
7) Age-matched lean mice

Data analyses: Differences in body weight and food intake were analyzed using Analyses of variance (ANOVA). To estimate the additive effect of the combination treatment, calculations were made in which the effects of the individual drug groups were summed relative to the vehicle control group. The summation that is bigger than the combined treatment group (positive) presents a supra-additive effect; the summation that equals the combined treatment group (zero) presents an additive effect; the summation that is smaller than the combined treatment group (negative) presents the combined drug effect that is less than the sum of the individual drug effects (Wellman et al., Pharm Biochem & Behav 75: 103-114, (2003)).

12. As can be seen from Exhibits 1, 2 and 3, the combination of AM 251 and phentermine produced an unexpected greater than additive, or supra-additive, inhibition of body weight gain and food intake than either treatment alone.

Discussion of Body Weight Loss Results for Exhibits 1 and 2:
By day 14, AM251 administration evoked dose-dependent body weight loss (3.7% BW loss with 1 mg/kg AM251; 7.4% BW loss with 3 mg/kg AM251). Significant differences between 1 and 3 mg/kg AM 251 in body weight (BW) loss were observed from day 4 onward. Phentermine at 10 mg/kg evoked significant body weight loss (4.2% BW loss). The combination of AM 251 and phentermine produced a greater inhibition of body weight gain than by either treatment alone. The combination of AM251 and phentermine treatment also showed a dose-dependent body weight loss with days of treatment. The

significant difference in body weight change appeared from day 4 through out the study. The low dose combination led to a 10% body weight loss while the higher dose combination yielded a 12.8% body weight loss (Fig 1). The summation value is zero for the first 3 days and positive for the rest of days of treatment with low dose of AM 251 combined with phentermine. The summation value is negative for the first 5 days and positive from day 6 onward with high dose AM 251 combined with phentermine (Fig 2). The data indicated that with days of treatment, the combination of AM 251 with phentermine tended to have a supra-additive effect on body weight loss. This supra additive effect was more evident in the low dose AM 251 combination treated group than in the high dose AM251 combination group.

Discussion of Food Intake Reduction Results for Exhibit 3:
AM251 reduced food intake in a dose-dependent manner, with the greatest effects occurring over the first 5 days of the study. 1 mg/kg AM251 reduced daily food intake on days 1 and 2. 3 mg/kg AM251 reduced daily food intake for 4 days. Moreover, on day 3, there was a significant difference of daily food intake suppression between 1 and 3 mg/kg AM 251 treatment groups. Phentermine reduced daily food intake for 4 days. The low dose combination reduced daily food intake for the initial 3 days of the study. The high dose combination suppressed daily food intake for 5 days. Significant inhibition of cumulative food intake relative to vehicle was observed in the 3 mg/kg AM251 treatment group (13.9% decrease in food intake) and in the two combination groups (AM251 at 1 mg/kg + phentermine and AM 251 at 3 mg/kg + phentermine; 16.0% and 21.9% decrease in food intake, respectively). There was no statistically significant effect of phentermine alone or AM251 at 1 mg/kg alone on cumulative food intake (4.3% and 5.5% decreased food intake, respectively). The data analyses indicated that the empirical combination of AM 251 with phentermine exerted a supra additive effect; there was a greater food intake reduction observed than the hypothetical summation of each individual drug alone. This was most evident in the low dose AM 251 and phentermine combination group.

13. Thus, in my opinion, one of ordinary skill in the art would have found it surprising and unexpected that the combination of AM251 and phentermine resulted in: 1) supra-additive changes in the reduction of body weight, and 2) supra-additive changes in the reduction of food intake.

14. Hyperlocomotion, or increased locomotor activity, is an undesirable side effect of phentermine administration in rodents as disclosed in Rowley et al., Synapse. 2000 Nov; 38 (2):167-76.

15. Exhibit 4 is a graph showing the unexpected decrease in locomotor activity for the combination of AM251 and phentermine relative to the locomotor activity of phentermine alone. The data for Exhibit 4 was obtained as follows:

Study Protocol: *Animals:* Male DIO C57BL/6N mice were fed with high fat diet and individually housed in a room with a 12 hr light photoperiod. Mice were housed in equipment that allowed for continuous monitoring of food intake and locomotor activity over a 14 day period. Mice were allowed ad libitum access to water and food. *Method:* Mice were conditioned orally with vehicle (5% tween, 0.25% methyl-cellulose) for 10 days before they were dosed with the drugs listed for Groups 1-4 below. Body weights and locomotor activity were monitored daily.

Groups (n = 8)
1) Vehicle
2) AM 251 @ 1 mg/kg
3) Phentermine @ 10 mg/kg
4) AM 251 (1) + Phentermine (10)

Data analyses: Differences in body weight and food intake were analyzed using Analyses of variance (ANOVA). To estimate the additive effect of the combination treatment, calculations were made in which the effects of the individual drug groups were summed relative to the vehicle control group. The summation that is bigger than the combined treatment group (positive) presents a supra-additive effect; the summation that equals the combined treatment group (zero) presents an additive effect; the summation that is smaller than the combined treatment group (negative) presents the combined drug effect that is less than the sum of the individual drug effects (Wellman et al., Pharm Biochem & Behav 75: 103-114, (2003)).

16. As can be seen from Exhibit 4, the locomotor activity of phentermine dosed alone unexpectedly and surprisingly decreased with the combination of AM 251 and phentermine.

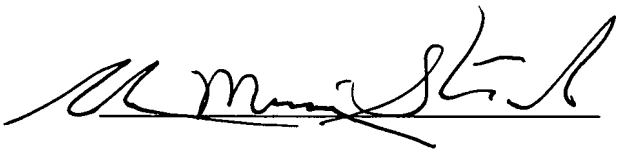
Discussion of Locomotor Activity Reduction Results for Exhibit 4:
Locomotor activity was unchanged by AM251 treatment but more than doubled relative to vehicle with phentermine treatment. The extent of the increased locomotion by phentermine was decreased when phentermine was co-dosed with AM251. (Exhibit 4). On days 6, 9, 12 and 13, the locomotor activity ($p < 0.05$) was decreased in the combination dosing group relative to phentermine alone.

17. Thus, in my opinion, one of ordinary skill in the art would have found it surprising and unexpected that dosing the combination of AM251 with phentermine ameliorated the hyperlocomotion observed with phentermine dosed alone.

18. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Full Name: Alison Merwin Strack

Signature: Date:



27-JUL-2007